Improving the Efficiency of Artificial Selection: More Selection Pressure With Less Inbreeding

Leopoldo Sanchez,*,[1] Miguel Angel Toro[†] and Carlos García*

**Departamento de Bioloxía Fundamental, Facultade de Bioloxía, Universidade de Santiago, 15706 Santiago de Compostela, Galicia, Spain and [†]Instituto Nacional de Investigaciones Agrarias, 28040 Madrid, Spain*

Manuscript received July 11, 1998
Accepted for publication December 14, 1998

ABSTRACT

The use of population genetic variability in present-day selection schemes can be improved to reduce inbreeding rate and inbreeding depression without impairing genetic progress. We performed an experiment with *Drosophila melanogaster* to test mate selection, an optimizing method that uses linear programming to maximize the selection differential applied while at the same time respecting a restriction on the increase in inbreeding expected in the next generation. Previous studies about mate selection used computer simulation on simple additive genetic models, and no experiment with a real character in a real population had been carried out. After six selection generations, the optimized lines showed an increase in cumulated phenotypic selection differential of 10.76%, and at the same time, a reduction of 19.91 and 60.47% in inbreeding coefficient mean and variance, respectively. The increased selection pressure would bring greater selection response, and in fact, the observed change in the selected trait was on average 31.03% greater in the optimized lines. These improvements in the selection scheme were not made at the expense of the long-term expectations of genetic variability in the population, as these expectations were very similar for both mate selection and conventionally selected lines in our experiment.

ARTIFICIAL selection brings genetic progress, but also increases the rate of inbreeding (Lush 1946; Robertson 1961), which results in inbreeding depression of the selected trait itself and fitness components such as fecundity and viability (Falconer and Mackay 1996). The problem has become more serious in present-day animal and tree breeding, in which short-term selection responses are maximized by the use of the animal model best linear unbiased predictor (BLUP) genetic evaluations. There is a consensus that BLUP is the best available method for genetic evaluation, as it increases the accuracy of selection by using all the available information from relatives in the evaluation of the selection candidates. However, the naive use of BLUP evaluation may also increase the inbreeding rate and the loss of genetic variability, because related individuals tend to be selected together, as they share most of their familial information (Belonsky and Kennedy 1988; Toro *et al.* 1988a). In fact, in small populations, the use of less family information may provide more response in the mid and longterm (Perez-Enciso and Toro 1992; Grundy *et al.* 1994). Other recent developments, such as the use of multiple ovulation and embryo transfer to increase female family size in cattle and the use of only ancestor's information in genetic evaluations to shorten the generation intervals (Nicholas and Smith 1983) tend also to speed the loss of genetic variability in selected populations.

In recent years, a considerable amount of work has been done on the design of strategies to optimize the use of genetic variability in artificial selection (reviewed in Toro and Perez-Enciso 1990). Most of these strategies try to maximize the selection response while at the same time imposing some restriction on the resulting loss of genetic variability in the population. This loss can be measured not only by the inbreeding coefficient, but also by the average coancestry coefficient, the founder equivalents, and the founder genome equivalents (see the review by Ballou and Lacy 1995). These strategies can be classified into three groups: (1) those manipulating the number of selected individuals (Villanueva *et al.* 1996) or their contribution to the next generation (Toro and Nieto 1984; Nieto *et al.* 1986; Wray and Goddard 1994; Meuwissen 1997); (2) those using a suboptimal selection criterion, giving less weight to family information (see Villanueva and Woolliams 1997 for the latest developments of this idea); and (3) those using a nonrandom mating system, such as minimum coancestry, factorial, or compensatory mating (see review by Caballero *et al.* 1996).

The mate selection method proposed by Toro and Perez-Enciso (1990) may include most of the desirable properties of the above methods. It consists of selecting, from the series of all possible mating sets between the selection candidates, that set maximizing the expected

Corresponding author: Carlos García, Area de Xenética, Departamento de Bioloxía Fundamental, Facultade de Bioloxía, Universidade de Santiago, 15706 Santiago de Compostela, Galicia, Spain.
E-mail: bfcarlog@usc.es

[1]*Present address:* Department of Forest Genetics and Plant Physiology, Swedish Institute of Agricultural Science, SE-901 83 Umeå, Sweden.

response in the next generation, under the restriction that the resulting inbreeding does not exceed a chosen limit. Two mating sets can be different because of the number of parents, the distribution of the contribution in descendants of these parents, or the actual matings between them. By choosing an optimum mating set, the method erases the distinction between selection and mating, as both kinds of decisions are taken simultaneously. The joint control of selection and mating could enable a noticeable short-term optimization of artificial selection schemes (Toro and Perez-Enciso 1990; Toro *et al.* 1991; Klieve *et al.* 1994; Nomura and Yonezawa 1994).

Mate selection and all the other optimization methods described above, with the exception of Nieto *et al.* (1986), have been tested to date only in computer simulation studies that were based on very simple infinitesimal, additive genetic models, which do not take proper account of inbreeding depression (Quinton *et al.* 1992). As this inbreeding depression is a manifestation of interactions such as dominance and epistasis, models that include only additive effects and covariates for inbreeding are rough approximations (Smith and Maki-Tänila 1990), and the computer simulation studies based on them cannot provide a final test of the performance of any new mating or selection scheme. An empirical assay with a real trait in a real population is needed to prove its usefulness.

In this work, we tested Toro and Perez-Enciso's (1990) mate selection method by applying it to a laboratory population of *Drosophila melanogaster*, and by measuring the selection pressure, the inbreeding depression, and the changes in genetic variability obtained.

MATERIALS AND METHODS

Selection lines were developed from a *D. melanogaster* laboratory population taken from the wild in Santiago de Compostela in 1992 and kept since then at 25° in a culture medium made up of 250 g whole corn flour, 180 g live baker's yeast, 18 g agar, 3 g ClNa, 3 g Nipagin, 33.3 ml ethanol, and 9.4 ml propionic acid in 2.2 liters water. The same conditions were used in this experiment. Every female in the selection lines laid eggs in a 23.7-cm^3 plastic vial.

The experiment had three nonsimultaneous replicates. Each one was started with 64 virgin pairs sampled from the base population. Two sets of four males and four females were taken from the progeny of every pair, and each set was used to start one selection line. The mate selection method was applied in one of them (OPT line), and a standard selection plan was applied in the other (REF line). We made six generations of selection in each line. The selected trait was pupa length, measured in arbitrary micrometer length units (mlu) by means of a micrometer introduced in one of the oculars of a binocular microscope. The females' fecundity was also measured, as the number of eggs laid by every selected female in its culture vial after 24 hr in replicates 1 and 2. In replicate 3, every selected female laid eggs on a black plastic rectangle that was covered on one of its sides by a layer of medium and introduced into the vial. After 48 hr, the eggs were counted and the layer of medium was divided in two pieces containing similar numbers of eggs, and each one was introduced in a different vial already containing 3 ml culture medium. Having two different vial effects for every female, we intended to measure better the variation between vials. The selection procedures used in each line were as follows:

REF selection line: The 256 (64 × 4) male and 256 female pupae assigned to this line were measured, and the longest 8 males and 64 females were selected. Each selected male was mated to a random sample of 8 selected females. The mating took place in two steps, each step comprising 4 adult females introduced into the male's vial for 24 hr. They were kept identified by clipping different combinations of their two posterior scutellar bristles. We measured 4 males and 4 females from every female's progeny and used these measures, along with all their ancestors' information, to obtain their BLUP genetic evaluations. Using this as a criterion, 8 males and 64 females were selected and the next generation established. The same procedure was followed for the remaining selection generations.

OPT selection line: In the same way as in the REF line, 4 males and 4 females from the progeny of 64 females were measured in every generation, but the selection procedure was different. In this line, we searched for the set of matings between the selection candidates that maximized the expected selection response in the following generation, under a restriction on the inbreeding increase. The allocation of matings can be treated as a linear programming problem (Jansen and Wilton 1985; Kinghorn 1987) by using an **X** matrix, where x_{ij} $\{i = 1, 256, j = 1, 256\}$ is a decision variable indicating whether male i and female j are ($x_{ij} = 1$) or are not ($x_{ij} = 0$) to be mated. Following the mate selection method, we tried to find, among all possible **X** mating matrices, the maximizing objective function,

$$\sum\sum x_{ij} (\hat{a}_i + \hat{a}_j)/2,$$

that is, the expected genetic gain in the next generation, in which $\hat{a}_i$ and $\hat{a}_j$ are the BLUPs for the breeding values of male i and female j. In this experiment, to save computational resources, we did not apply the method to the whole array of 256 × 256 possible matings, but made a preselection of the 32 males and 64 females having the highest genetic evaluation and selected a set of matings between them. The objective function was subjected to the following restrictions:

1. $\Sigma\Sigma x_{ij} = 64$
2. $\Sigma x_{ij} = 0$ or 1 (for every j)
3. $\Sigma x_{ij} \leq 12$ (for every i)
4. $\Sigma\Sigma x_{ij} f_{ij}/64 < (F + \delta F)$,

where F and f_{ij} are the average population inbreeding in the previous generation and the coancestry coefficient between the ith male and the jth female, and δF is the maximum additive increase in inbreeding allowed per generation. We used an additive increase in F as the restriction because we wanted a criterion that was simple and did not depend on the changes in inbreeding in the previous generations. Thus, mate selection maximized the expected response while respecting some reproductive restrictions (1 to 3) and a restriction (4) on the inbreeding increase, and did it by using nonrandom mating, a variable number of sires, and a variable mating ratio. In all replicates, the solution matrix **X** was obtained with linear programming computer programs that were written in the computer center of the Instituto Nacional de Investigaciones Agrarias (Madrid) following the algorithms given by Land and Powell (1979).

The magnitude of the restriction on the inbreeding increase was not constant throughout the experiment. In replicate 1 and in generations 1–4 of replicate 2, the limit value for δF was 0.03 per generation, which was fixed by using as reference

the increase in F per generation that was predicted for the REF line by the Robertson (1961) effective population number expression. This value of 0.03 is higher than those typically used in large populations of livestock, which are ~0.01 (Wray and Goddard 1994), but was appropriate for a smaller and intensively selected population as was used in this experiment. From generation 5 of replicate 2, we changed our procedure to fix the maximum F increase allowed in the OPT line, because in that generation the observed F in the REF line was not only less than the average value predicted with the Robertson (1961) expression, but even less than that observed in the OPT line, which was respecting the restriction fixed on its F increase. This can happen in practice, as a particular population can be above or below its expected F value. To avoid this, we first set up the random matings in the REF line, and then calculated the expected F increase for these particular matings. This value was used as a reference to fix the maximum increase in F that was allowed in the OPT line in that generation. We kept using this new procedure in the rest of the experiment (*i.e.*, in generations 5 and 6 of replicate 2 and in all of replicate 3). Thus, we made sure from that moment that the OPT line had lower inbreeding than its corresponding REF line.

Data analysis: We obtained two genetic evaluations for every individual. The first, EBV1, was an animal-model BLUP based on all the information available on every animal at the time of making selection decisions, and included its phenotypic value and that of its sibs and ancestors. It was used as a selection criterion. The second, EBV2, was the BLUP evaluation used in the final analysis of the results, and was obtained by using all the information available at the end of the experiment, which included all the individual's descendants in addition to the information used for EBV1. The genetic evaluations for the OPT and REF lines in a given replicate of the experiment were obtained with a single execution of the corresponding program as both lines descended from the same set of founders, and it was possible to include them in a single genealogical file.

As the experiment advanced, we used more complete theoretical models and more recent computer programs to obtain the EBV1s. In replicates 1 and 2 we used JAA, the univariate BLUP program by Misztal and Gianola (1987), and in replicate 3 we obtained these EBV1s with DFREML(UNI), the univariate version of the derivative-free program for BLUP genetic evaluation and variance component estimation by Meyer (1991, 1993). This was also used to obtain the EBV2s in all replicates.

We obtained additional estimates for the EBV2 and variance components using a Bayesian approach. There are multiple advantages to this approach: use of prior information (when available), elimination of nuisance parameters, exact finite sample analyisis and integrated estimation, prediction, and decision (Gianola *et al.* 1989). Markov chain Monte Carlo methods such as Gibbs sampling allow us to draw Bayesian inferences about genetic parameters and responses (Wang *et al.* 1994). The MTGSAM set of programs by Van Tassell and Van Vleck (1996) accomplished Gibbs sampling analysis for multitrait models. In our case the model included pupa length and fecundity. We assumed flat prior distributions for fixed effects and normal distributions for all random effects in the model, including residuals. For the genetic effects it is additionally assumed that there is a known covariance structure corresponding to the numerator relationship matrix. Finally, we also assigned flat prior distributions for all (co)variance components. The Gibbs sampler was run once for every replicate in the experiment, with 500,000 iterations in each run, the first 2000 iterations being discarded ("burn in"). As a consequence of the use of conditional probabilities in each step of Markov chains, there is an autocorrelation between their successive elements, so that an original chain can be reduced to a shorter one having lower autocorrelation without loss of information. We saved a sample of the parameters of interest every 500 iterations, so that we had 1000 saved samples per replicate. We obtained Gibbs samples for (co)variance components, breeding values, and inbreeding depression effects for both characters, while samples for the slope of the regression of the mean EBV on generation number were obtained for pupa length only.

A summary of the computer programs and theoretical models used in the data analysis can be seen in Table 1. We used a heritability of 0.3 for pupa length in the analyses made in replicate 1 with JAA. The programs DFREML and MTGSAM use the actual data set to estimate the parameters required to obtain the genetic evaluations.

The theoretical model used for the data analysis included the effect of generation and, as long as the data reflect the true genetic situation, they allow us to separate realized genetic changes from environmental changes between generations within a selection line. In any case the aim of the experiment was to compare the OPT and REF selection lines; the generation environmental effects would not affect the comparison, because the OPT and REF lines in the same replicate were maintained simultaneously and therefore shared these effects.

We measured the effect of mate selection on response as the difference in phenotypic response between the OPT and REF lines. We used the expression given by Aggrey *et al.* (1995) for the expected variance of this difference between two selection lines, which takes into account both drift variance and measurement error,

$$s_R^2 = \frac{2\sigma_A^2}{N_e}\left\{t + \frac{4}{(1+\beta)h^2}\left[c^2 + \frac{\beta p_m}{1+\alpha}(1 - c^2 - h^2/2)\right]\right\},$$

where σ_A^2 is the additive variance, N_e the effective population size, which was calculated taking into account the different numbers of male and female parents, t is the number of generations, p_m is the proportion of males selected, α the number of females scored for every male, β the number of females selected for every male, h^2 the trait's heritability, and c^2 the environmental correlation between full sibs. We used the estimated variance for environmental vial effects as the environmental covariance between full sibs. However, this expression is but an approximation that does not take into account all the effects of directional selection on genetic variance (Aggrey *et al.* 1995). To complement this test and to obtain a more empirical value for the standard deviation of selection response, we also carried out 100 runs of a computer simulation of selection lines with the features of the REF lines in our experiment.

We studied nonrandom mating in the selection lines by means of Wright's F statistics (Wright 1969),

$$(1 - F_{IT}) = (1 - F_{ST})(1 - F_{IS}),$$

where F_{IT} is the average inbreeding of animals born in a given generation and was calculated with the genealogical information, F_{ST} is the average coancestry coefficient between the sires and dams of all possible mates and provides a measure of the contribution of limited population size to inbreeding, and F_{IS} measures that of nonrandom mating.

In addition to inbreeding coefficients, we used methods based on the genetic contributions from founders and probabilities of gene loss to study the maintenance of genetic variability in the experiment. First, we calculated the "number of founder equivalents" (Lacy 1989), which is a measure of the balancedness of the contributions from the different founders, *i.e.*, the 64 males and 64 females used to start every replicate,

TABLE 1

Statistical models and computer programs used in the data analysis

	Replicate 1	Replicate 2	Replicate 3
EBV1 model	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{Z}_1\mathbf{s} + \mathbf{e}$	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{Z}_1\mathbf{s} + \mathbf{e}$	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{X}_3\boldsymbol{\beta}_3 + \mathbf{Z}_1\mathbf{s} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$
CP	JAA	JAA	DFREML(UNI) v2.1
EBV2 model	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{X}_3\boldsymbol{\beta}_3 + \mathbf{Z}_1\mathbf{s} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{X}_3\boldsymbol{\beta}_3 + \mathbf{Z}_1\mathbf{s} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$	$\mathbf{y} = \mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{X}_3\boldsymbol{\beta}_3 + \mathbf{Z}_1\mathbf{s} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$
CP	MTGSAM	MTGSAM	MTGSAM

The matrix **y** contains the phenotypic measures, the **X**'s are design matrices, the **β**'s, vectors of fixed effects, with subindices 1 for the effect of sex (not considered for fecundity), 2 for the effects of the generation number, and 3 for the inbreeding depression covariable; **s** is the vector of breeding values, **p** is a vector of random environmental vial effects, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are their corresponding design matrices, and **e** is the error vector. See the text for information about the computer programs. CP, computer programs.

$$f_e = 1 / \sum_{i=1}^{M+F} c_i^2,$$

where M and F are the numbers of male and female founders and c_i is the total contribution of founder *i* to the gene pool of the population or the probability that a gene randomly sampled in this population originates from founder *i*. It was calculated as

$$c_i = \sum_{j=1}^{N} (a_{ij}/N),$$

where *a(i,j)* is the additive relationship coefficient between the founder *i* and the current animal *j* and *N* the number of animals of the current population ($\Sigma\, c_i = 1$). The sum of c_i^2 can be calculated in every generation, and after several generations, the distribution of *c* stabilizes, and then the sum of c_i^2 predicts the asymptotic rate of inbreeding as $\Delta F = (1/4)\ \Sigma\ c_i^2$ (Wray and Thompson 1990; Woolliams and Thompson 1994). We observed this stabilization at the end of our experiment, and used the sum of c_i^2 in the last generation to calculate the asymptotic rate of inbreeding.

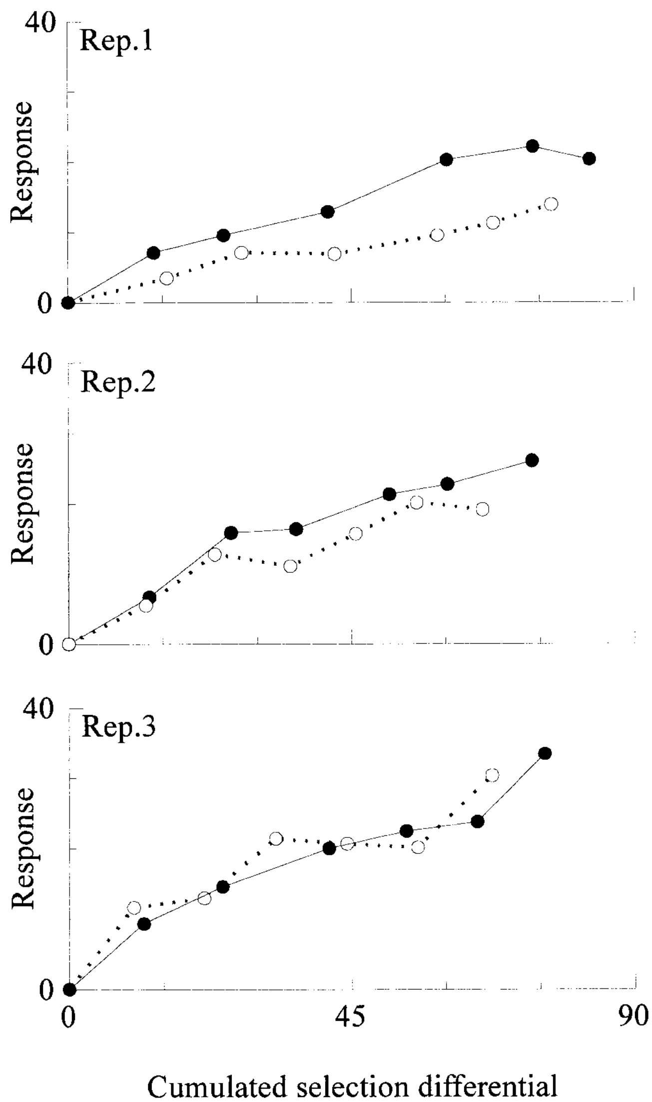

Figure 1.—Phenotypic selection response and phenotypic cumulated selection differentials for pupa length in mlu in the OPT (continuous) and REF (broken) selection lines.

We calculated also a direct measure of the amount of genetic variability remaining in each line at the end of the experiment. This was the "founder genome equivalent" (Lacy 1989),

$$f_g = 1/\sum_{i=1}^{M+F}(c_i^2/r_i),$$

where r_i is the probability of a given gene from founder i to be retained in the population of descendants. It was calculated with the gene drop computer simulation technique (MacCluer *et al.* 1986), which simulates the transmission of a gene from every founder parent through the genealogy, assuming that the probability of a parent's gene being transmitted to a descendant is 50%. The value of r_i is taken as the proportion of runs in which the gene is present in the descendants' generation. The founder genome equivalent may be defined as the number of equally contributing founders, with no random loss of founder alleles in the offspring, that would be expected to produce the same genetic diversity as in the population under study. In contrast with the founder equivalent, it takes into account the effect of genetic drift and population bottlenecks on gene loss (Lacy 1989).

RESULTS

Selection pressure and inbreeding: The OPT lines showed increases in phenotypic selection differential of 7.9, 11.9, and 12.4% over the corresponding REF lines in replicates 1, 2, and 3, respectively (Figure 1). The increases of EBV2 selection differential were 28.4, 31.0, and 9.0% in the same replicates. A Wilcoxon signed rank test comparing the phenotypic selection differentials applied in the OPT and REF lines in every replicate and generation found a significant advantage for the OPT lines (18 observations, $S = 44.5$, $P < 0.027$, one-tailed test). This increase in selection pressure was accompanied by a relative reduction in inbreeding (Figure 2). The restriction on average inbreeding coefficient resulted also in more homogeneous inbreeding coefficients in the OPT line (Figure 2). Reducing the variance in F is very desirable in practice, because the number of highly inbred individuals is also reduced.

A summary of the MTGSAM-estimated parameters can be seen in Table 2. All the results obtained with the DFREML analysis were very similar to those found with MTGSAM, so that they are not shown. The heritability estimates were in accordance with published values for fecundity (Rose and Charlesworth 1981) and body size (Coyne and Beecham 1989) in this species. The estimates for the genetic correlation between pupa length and female fecundity were negative, in contrast with the positive values obtained in other experiments (Robertson 1957; Mackay 1985). It is expected, however, that estimates of fitness and its resolution into components depend heavily on the experimental conditions of measurement (Mackay 1985).

The significantly increased selection differentials in the OPT lines would result in increased responses in pupa length, which had a medium to high heritability in our population, and in fact the average pupa size was greater in the three OPT lines at the end of the experiment (Figure 1). But given the limited size of the experiment, it was difficult to detect as significant these observed differences. We had, however, some evidence of greater selection response in the OPT lines. First, the Gibbs sampling analysis of selection response per

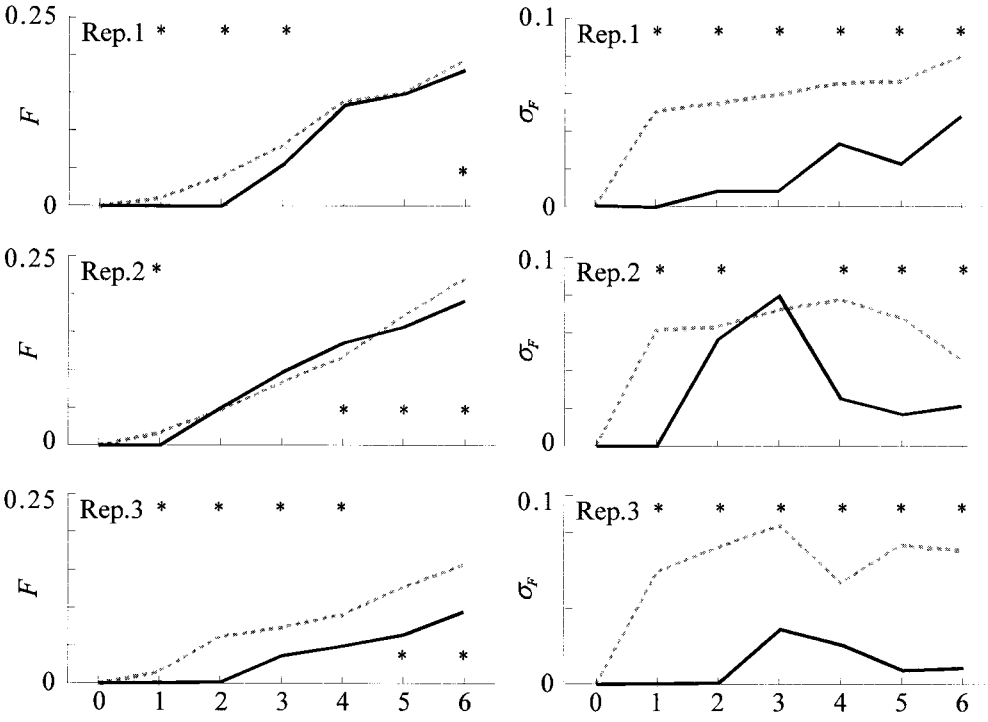

Figure 2.—Inbreeding coefficients. The continuous and broken lines trace the mean and standard deviation of inbreeding coefficients in the OPT and REF selection lines, respectively. The generations in which there was a significant ($P < 0.05$) difference between means (t-test) or variances (F-test) are marked with asterisks.

TABLE 2

Gibbs sampling estimates (± standard error in the marginal posterior distribution)

	Replicate 1	Replicate 2	Replicate 3
$h^2(1)$	0.378 ± 0.067	0.304 ± 0.071	0.399 ± 0.051
r_G	−0.091 ± 0.199	−0.312 ± 0.213	−0.403 ± 0.199
$h^2(2)$	0.265 ± 0.076	0.291 ± 0.090	0.209 ± 0.074
$c^2(1)$	0.301 ± 0.027	0.289 ± 0.030	0.229 ± 0.019
$c^2(2)$	0.007 ± 0.010	0.007 ± 0.008	0.024 ± 0.025

Estimates of heritabilities (h^2) and of ratios: variance of vial effect/phenotypic variance (c^2) for the characters' pupa length in micrometer length units (1) and fecundity as number of eggs laid (2), and genetic correlation (r_G) between them.

generation found a significant advantage for the OPT lines in replicates 1 and 2 (Figure 3). Second, the across-replicates average difference in total phenotypic response between OPT and REF lines (Table 3) was found to be significant when compared with its expected standard deviation, as calculated with the Aggrey *et al.* (1995) expression. This expected standard deviation was 2.14 mlu, so that the average difference of 5.48 mlu would be equal to 2.56 standard deviations, and therefore significantly greater than zero ($P < 0.01$) in a one-sided test for a normal distribution. The standard deviation of total response obtained with 100 simulations resulted also in a significant difference between the OPT and REF lines. It was equal to 2.86 mlu, so that the expected standard deviation of the difference between two means of three lines each would be $2.86 \times \sqrt{2/3} = 2.34$ mlu. This value was used to compare the observed average difference of 5.48 mlu with zero in a one-sided t-test ($t_{99} = 2.35$, $P < 0.025$).

It is also possible to do a nonparametric test for the basic result in this experiment: the three OPT lines attained a higher average pupa length and a lower inbreeding coefficient than their corresponding REF lines. Under the null hypothesis of no effect of mate selection, the probability of an OPT line being above its corresponding REF line in pupa length would be 0.5, and that of being below it in inbreeding would also be 0.5. Assuming that the results for both variables are independent, the probability of obtaining such a result at random would be $(0.5)^6 = 0.0156$. In fact these two variables were not independent in our experiment, as there was a positive correlation between them. Within treatments, the lines and generations with more response tended to have more inbreeding, and therefore the described test would be conservative.

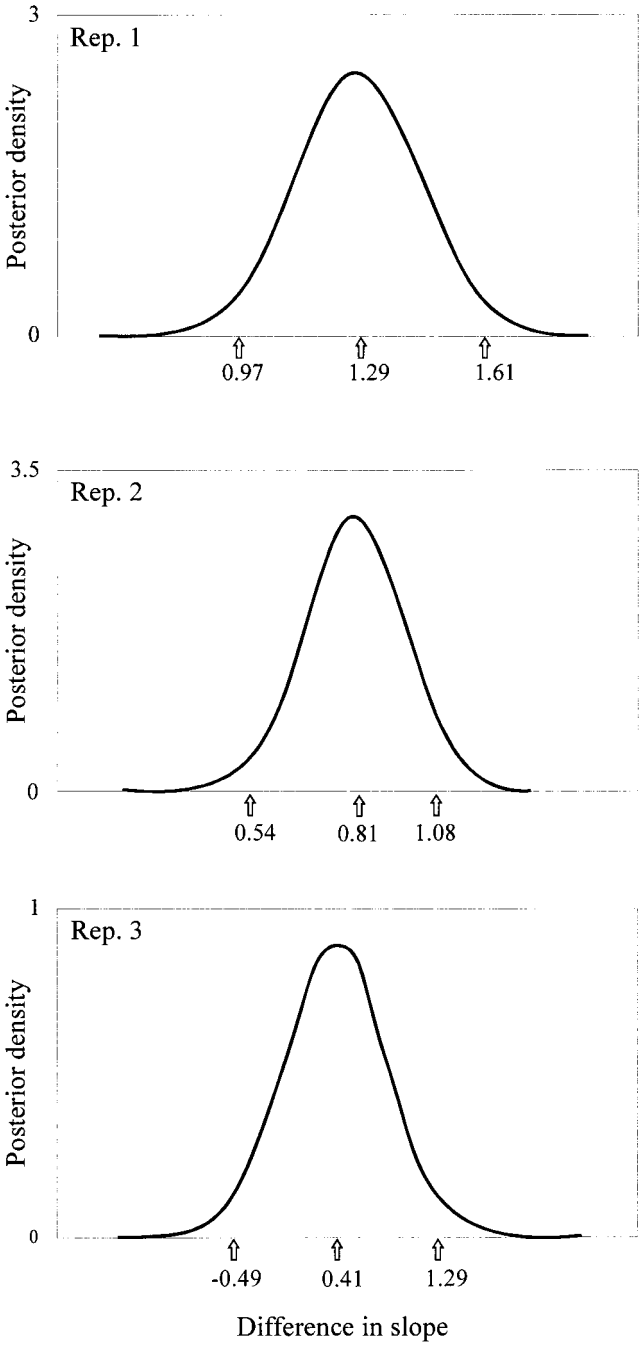

Figure 3.—Gibbs sampling analysis of selection response. Represented are the Gibbs sampling marginal posterior distributions of the difference between the slopes of the regression of pupa length average EBV2 in mlu on generation number in the OPT and REF lines. In the horizontal axis are the distribution mean and the two values defining the 95% highest posterior density intervals. These values mark a 95% two-tailed confidence interval for the mean.

Mate selection effects on mating structure: The different mating schemes used in the OPT and REF lines caused differences in their total number of male parents and family size variance. These differences can be seen in Figure 4, in which the last generation of selection is given as an example. The differences were smaller in the first generations of the experiment, because there were still few related individuals in them, and the best evaluated ones tended to be selected in both lines. The resulting genealogical structures could have resulted in

TABLE 3

Cumulated selection response in mlu in generation 6

	Replicate			Average difference
	1	2	3	
Phenotypic response				
OPT	20.26	26.07	33.36	5.48
REF	13.79	19.14	30.32	
EBV2 response				
OPT	29.25	20.99	29.49	5.11
REF	22.92	15.10	26.38	

differences in the accuracy of BLUP genetic evaluations, and therefore in selection response. The Gibbs sampling produces a complete set of genetic evaluations for all the genealogies in every iteration, and thus we had a posterior distribution of estimated breeding values for every individual in the experiment. We used the within-individual variance in EBV1 as a measure of the precision of its genetic evaluation. We compared the average of these variances between lines by generation with *F*-tests, but found no consistent advantages for any line. However, in the *F*-tests that were significant, the OPT lines had a disadvantage in precision more often than the reverse (not shown).

The analysis of nonrandom mating revealed that there was some avoidance of mating between relatives in the OPT lines, as the F_{IS} were always negative in them (Figure 5). The F_{ST} for these lines were higher in replicates 1 and 2, but this could be due to their smaller average census number. On average along the experiment, the census numbers in the OPT lines were 85, 96, and 97% of that in the REF lines in replicates 1, 2, and 3. The relative reduction in census in replicate 1 was greater than the increase in F_{ST} ($0.85 \times 1.09 = 0.93$), and the reverse happened in replicate 2 ($0.96 \times 1.07 = 1.03$).

The reduction in census number in the OPT lines could be related to a negative genetic correlation between pupa length and fecundity (Table 2) and also to the negative genetic correlation between body size and larval viability found by Partridge and Fowler (1993) in this species. If the OPT lines had greater increases in pupa length, they would have suffered stronger negative correlated responses in fecundity and viability, thus failing more often to produce the required number of adult descendants from the selected individuals.

The observed avoidance of mating between relatives did not clearly result in disassortative mating in the OPT lines. We detected some significant mate correlations, positive and negative, for the EBV2 in both OPT and REF lines, but the between-line differences in correlation were not consistent among generations (Figure 5).

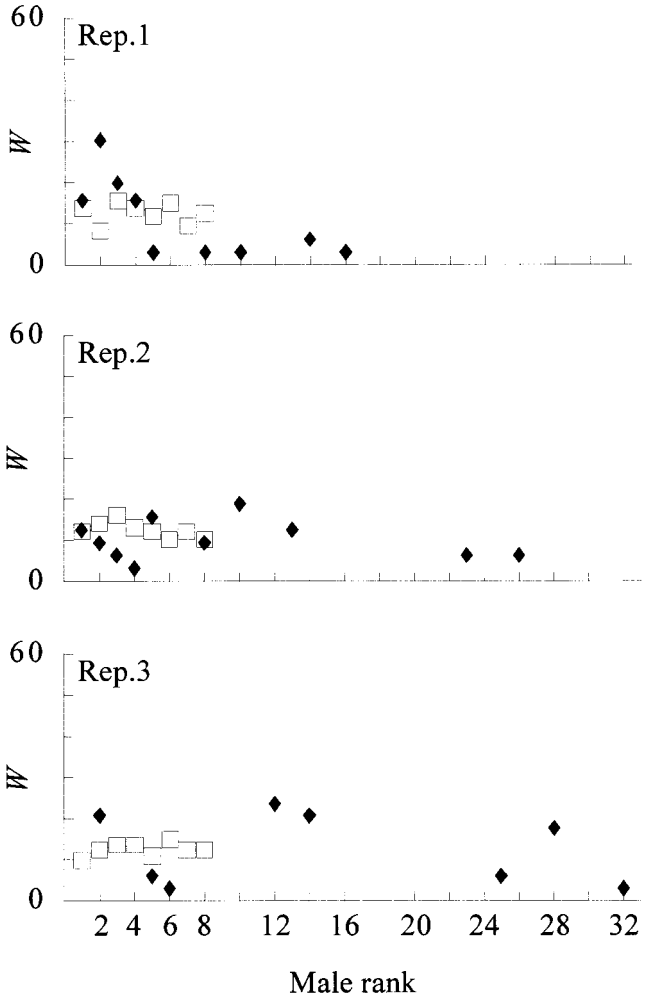

Figure 4.—Male contributions in offspring in generation 6. The contribution by every selected male in the OPT (◆) and REF (□) lines is expressed as a percentage *W* of the total number of descendants. The males are ordered according to their EBV1 ranking position, from the highest evaluation (1) to the lowest (32).

Inbreeding depression: The effect of inbreeding on fecundity was negative for the three replicates (Figure 6). There was an average reduction of 0.31 eggs laid (0.96% of the mean) with every 1% increase in *F*, and this indicates that the OPT lines, which had less inbreeding, also had less inbreeding depression. However, this reduction in inbreeding depression was not enough to compensate for the negative correlated responses in fecundity and viability to the selection applied on pupa length, and as seen above, the census number in the OPT line was on average somewhat lower than in the REF line.

The results for pupa length were more unexpected, as the effect of the inbreeding coefficient on this trait was negative and significant in replicate 3, but positive and nonsignificant in replicates 1 and 2 (Figure 5). On average we estimated a 0.01% increase in pupa length with every 1% increase in *F*. Positive effects of inbreed-

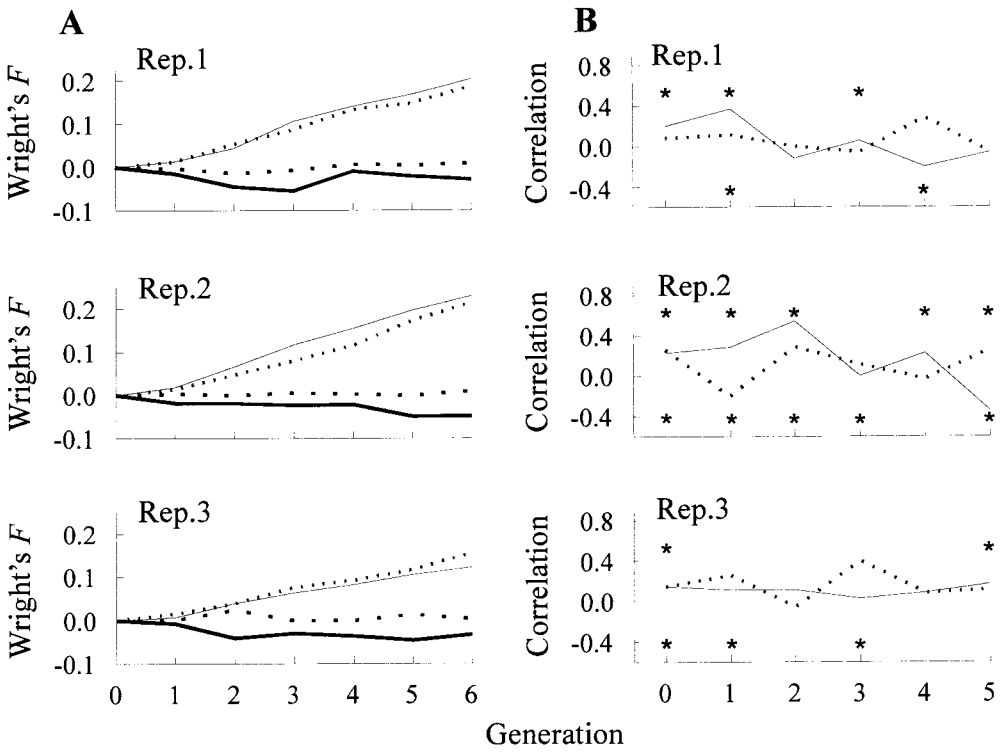

Figure 5.—Measures of the degree of nonrandom mating in OPT (continuous) and REF (discontinuous) selection lines. (A) Wright's *F*-statistics. Represented are F_{IS} (boldface lines) and F_{ST} values. (B) Correlation in EBV2 between mating males and females. The asterisks mark correlations significantly ($P < 0.05$) different from zero in REF (bottom row of asterisks) and OPT lines (top row of asterisks).

ing on pupa size have also been found in Tribolium (Groen *et al.* 1995a). We have no explanation for these apparent differences between replicates. It is known that the use of genealogical information does introduce biases in the estimation of the inbreeding depression in characters under selection (Burrow 1993; Groen *et al.* 1995b), so that our inbreeding depression measures for pupa length are less reliable than those obtained for fecundity. However, it is not obvious to us how different biases could have been introduced in replicate 3 than in replicates 1 and 2. Perhaps the fact that replicate 3 had a different treatment of egg laying and a different theoretical model for its analysis, or that it had a lower final inbreeding coefficient (Figure 2), could be relevant for the introduction of some differential bias between replicates. In any case, the OPT lines' advantage in pupa length cannot be explained by a lesser inbreeding depression caused by their lower inbreeding coefficients, as the estimates of the effect of inbreeding on the character were positive in replicates 1 and 2.

Pedigree analysis and long-term genetic variability expectations: Mate selection could be considered as a method to optimize artificial selection in the short term, as all the restrictions considered in it refer only to the next generation (De Boer and Van Arendonk 1994). However, it is known that often most gene losses in a population occur in the first generations of selection, so that the maintenance of genetic variability is not a long-term issue only, and it makes sense to study the effect of selection decisions taken at the beginning of the selection plan (Verrier *et al.* 1994). In accordance with this, we found that the distribution of genetic contributions from founders, measured as the number of founder equivalents, stabilized in the last generations of the experiment (Table 4). As explained in materials and methods, this stabilization made it possible to use the genetic contributions from founders observed in generation 6 to calculate the asymptotic rates of inbreeding. These were very similar for the OPT and REF lines, and there was even an overall advantage for the OPT lines (Table 5). In the case of the OPT lines, the asymptotic rates of inbreeding should be taken as measures of the amount of genetic variability remaining at the end of the experiment, instead of true predictions of their long-term behavior, because the Wray and Thompson (1990) method to calculate them assumes random mating, and of course, there was no random mating in the OPT lines.

The advantage found for the OPT lines was reversed in the next measure of genetic variability, the founder genome equivalent (Table 5). This difference in results could be explained by the OPT lines' average inferiority in census number, because, as seen in materials and methods, the founder genome equivalent is sensitive to population bottlenecks. However, on average the founder genome equivalent of the OPT lines was less reduced than their census number, and perhaps this could be taken as a sign of better management of genetic variability in the OPT lines. Finally, the population average coancestry at the end of the experiment was overall very similar in both kinds of line. Thus, when taking all

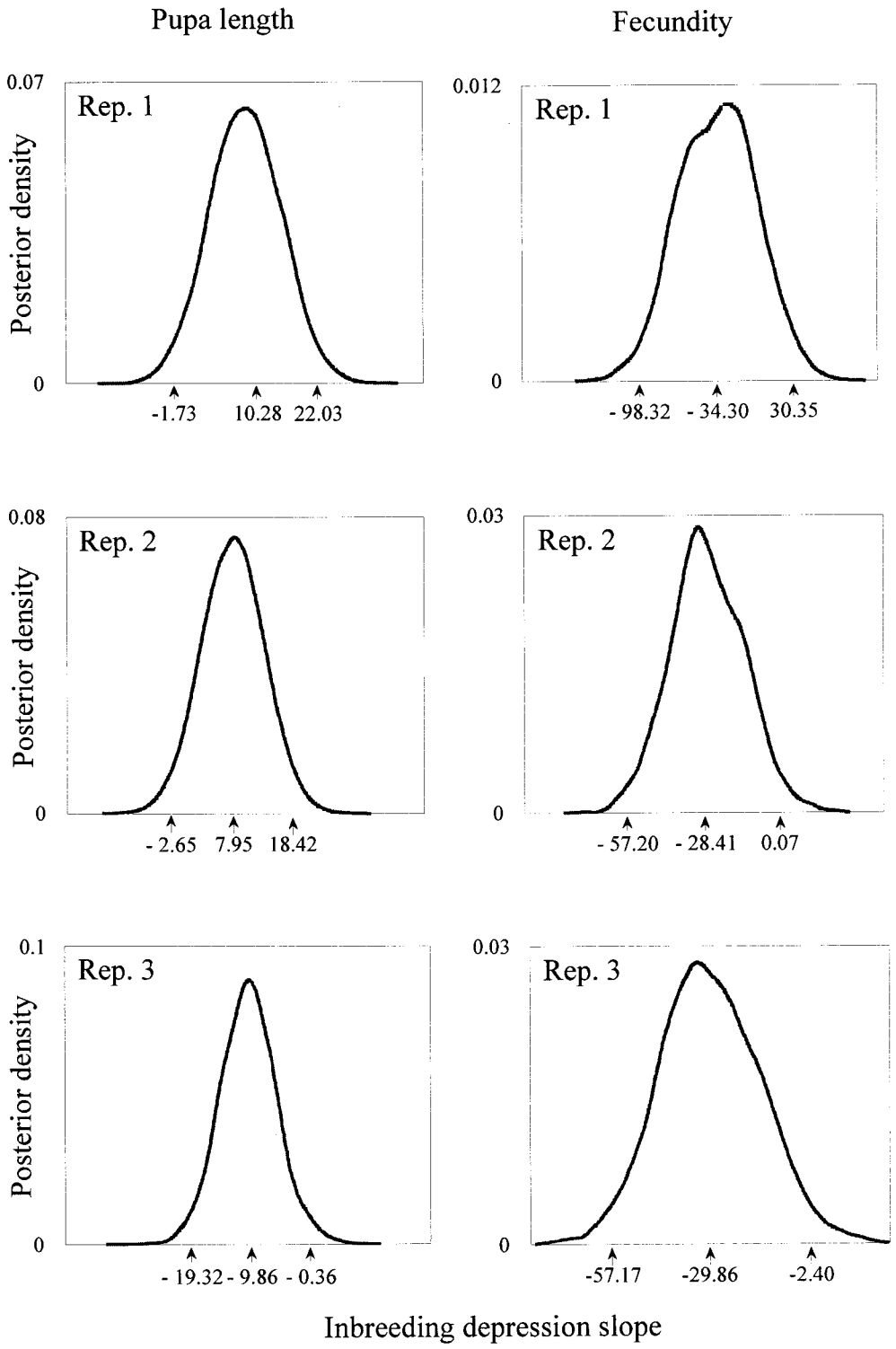

Figure 6.—Inbreeding depressions. Represented are the Gibbs sampling marginal posterior distributions of the slopes of the regression of phenotype for pupa length (in mlu) and fecundity (in number of eggs laid per female) on inbreeding coefficient, measured from zero to one. In the horizontal axis are the distribution mean and the two values defining the 95% highest posterior density intervals. These values mark a 95% two-tailed confidence interval for the mean.

measurements together, there was no clear evidence of a worse long-term management of genetic variability by mate selection.

DISCUSSION

The experiment showed that it is possible to improve the design of artificial selection schemes, as the mate-selected lines had at the same time an increased selection differential and a reduced rate of inbreeding. The advantages of reducing inbreeding refer not only to a better use of the genetic variability available in the base population and to a reduced inbreeding depression in the selected trait, but also to a reduced depression of fitness-related traits, which may be at present the most serious drawback of the increase in inbreeding in do-

TABLE 4

Number of founder equivalents by generation

		Generation						
Replicate	Line	0	1	2	3	4	5	6
1	OPT	120	17.19	12.56	10.81	10.15	10.38	10.48
	REF	120	11.83	10.74	10.65	10.63	10.78	10.85
2	OPT	106	16.35	10.53	9.87	10.39	10.62	10.71
	REF	106	13.26	13.12	10.85	10.17	8.39	8.22
3	OPT	112	21.73	16.01	15.55	15.26	14.96	15.20
	REF	112	19.15	16.04	13.21	14.43	14.61	14.31

mestic populations (Meuwissen and Woolliams 1994; Brisbane and Gibson 1995). These fitness-related traits are not often subjected to direct selection due to their low heritability, but have nevertheless great economic importance.

In our results, most of the OPT lines' reduction in inbreeding was obtained in the first generation of selection by avoiding sib mating. In the remaining generations, mate selection maintained the inbreeding difference while increasing the selection differential in the OPT lines. But this situation was a by-product of the mate selection ability to obtain a considerable lag in inbreeding coefficient at the start of the experiment, rather than an intrinsic incapacity to control inbreeding in later generations. For example, the OPT line had lost its initial advantage in inbreeding in generation 3 of replicate 2 and had a higher F than the REF line, for reasons explained above. In that situation, mate selection made it possible to increase the emphasis put on inbreeding, and still obtain a higher cumulated selection differential and a lower F in the last generation of this replicate. Thus, mate selection could generate an advantage in selection and inbreeding in generations different from the initial one. This highlights the ability of mate selection to trade to some extent selection differential for inbreeding coefficient, while producing a final result that is better in both respects than a conventional selection line. It is flexible also in applicability, and depending on the restrictions involved, it could be used to optimize a selection scheme either hierarchical or factorial, with a variable number of males, females, or offspring per mating, or with overlapping generations. Furthermore, different restrictions can be used in different generations or years (Toro *et al.* 1991).

TABLE 5

Measures of the genetic variability remaining in generation 6

	Replicate			Average ratio[a]
	1	2	3	
Asymptotic rate of inbreeding				
OPT	0.024	0.023	0.016	0.900
REF	0.023	0.030	0.018	
Founder genome equivalent				
OPT	3.989	3.530	5.731	0.951
REF	4.294	4.135	5.350	
Average coancestry[b]				
OPT	0.195	0.228	0.134	1.009
REF	0.180	0.221	0.147	
Average inbreeding[c]				
OPT	0.184	0.192	0.095	0.806
REF	0.194	0.221	0.158	

[a] The across-generations average of ratios: OPT line values / REF line values.

[b] The population average coancestry between males and females.

[c] The average inbreeding coefficient in each line in generation 6 is given for comparison.

The improved management of inbreeding depression provided by mate selection could permit reductions in census number in a selection nucleus in which very complicated or expensive selection criteria are measured. It could also be useful for the genetic conservation of rare breeds or species. Templeton and Read (1984) proposed applying artificial selection on fitness-related traits in small populations, to eliminate deleterious recessive genes and thus reduce the inbreeding depression. This selection should be accompanied by a restriction on the inbreeding rate, because too rapid increases in inbreeding could result in the loss of the population. Mate selection would be a good method to implement these conservation plans, as it enables control of the relative weight given to selection and inbreeding in every generation.

Among the optimizing methods of artificial selection that have been proposed to date, mate selection is the most short-term one, because it is the only method controlling simultaneously the selection and mating processes, and thus makes it possible to exert maximum control on the next generation results. Mate selection does not directly control the selection results beyond the next generation, but we found that its long-term

maintenance of genetic variability is not clearly worse than in a standard selection line, and concluded that it does not minimize the short-term inbreeding depression at the expense of long-term genetic variability. This result is consistent with that found by Toro *et al.* (1988b) in a computer simulation study using phenotypic selection, in which the mate selection lines had less inbreeding than the standard, randomly mated selection lines after 30 generations.

In any case, a general comparison of the different methods proposed to optimize the long-term results of selection plans is still lacking, even under computer simulation. This comparison could be difficult to carry out, because its results could depend on the particular selection scheme, the genetic model and parameters assumed, the time horizon objective, and the possible future use of the selected lines in crosses (Quinton and Smith 1995; Caballero *et al.* 1996). In addition, new methods might be developed in the future. For example, it would be possible to use simultaneously restrictions on the inbreeding in the next generation and on some prediction of the expected amount of genetic variability after *t* generations (as those described in Woolliams and Thompson 1994; Wray *et al.* 1994; Santiago and Caballero 1995). This would combine the short-term minimization of inbreeding depression provided by mate selection with a more direct long-term control of genetic variability (Wray and Goddard 1994). These two kinds of restrictions are not intrinsically antagonistic, and depending on the relative weight given to each, the emphasis on short- or long-term results could be varied. Perhaps the main difficulty in developing such a method lies in the combination of the nonrandom mating that is used in mate selection and the prediction of the properties of the population in future generations, because, as explained above, the present-day methods used to make these predictions assume random mating or some rather restricted circumstances.

We conclude that the efficiency of artificial selection plans can be improved, and that the advantages obtained by doing so may be big enough to be detected in rather small selected populations. However, no definitive general method seems to have been proposed up to date, and this remains a promising area of research that might provide many tangible benefits in animal and plant breeding.

We thank R. Rekaya for his help with data analysis. This work was funded by the Comisión Interministerial de Ciencia y Tecnología, Spain, with grant AGF92-1017-C03-02. Leopoldo Sánchez was supported by a predoctoral fellowship from the Xunta de Galicia.

LITERATURE CITED

Aggrey, S. E., C. Y. Lin and K. M. Cheng, 1995 Size of breeding populations required for selection programs. Theor. Appl. Genet. **91:** 553–556.

Ballou, J. D., and R. C. Lacy, 1995 Identifying genetically important individuals for management of genetic diversity in pedigreed populations, pp. 76–111 in *Population Management for Survival and Recovery*, edited by J. D. Ballou, M. Gilpin and T. J. Foose. Columbia University Press, New York.

Belonsky, G. M., and B. W. Kennedy, 1988 Selection on individual phenotype and best linear unbiased predictor of breeding value in a closed swine herd. J. Anim. Sci. **66:** 1124–1131.

Brisbane, J. R., and J. P. Gibson, 1995 Balancing selection response and rate of inbreeding by including genetic relationships in selection decisions. Theor. Appl. Genet. **91:** 421–431.

Burrow, H. M., 1993 The effects of inbreeding in beef cattle. Anim. Breed. Abstr. **61:** 737–751.

Caballero, A., E. Santiago and M. A. Toro, 1996 Systems of mating to reduce inbreeding in selected populations. Anim. Sci. **62:** 431–442.

Coyne, J. A., and E. Beecham, 1989 Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. Genetics **117:** 727–737.

De Boer, I. J. M., and J. A. M. Van Arendonk, 1994 Additive response to selection adjusted for effects of inbreeding in a closed dairy cattle nucleus assuming a large number of gametes per female. Anim. Prod. **58:** 173–180.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Longman, London.

Gianola, D., S. Im and F. W. Macedo, 1989 A framework for prediction of breeding value, pp. 210–238 in *Advances in Statistical Methods for Genetic Improvement of Livestock*, edited by D. Gianola and K. Hammond. Springer-Verlag, Berlin.

Groen, A. F., W. F. Fikse and P. J. Berger, 1995a Effects of data structure and selection on estimated inbreeding trend and depression in experimental *Tribolium castaneum* lines. Paper presented at the 46th Annual Meeting of the European Association for Animal Production, Prague 1995.

Groen, A. F., B. W. Kennedy and J. J. Eissen, 1995b Potential bias in inbreeding depression estimates when using pedigree relationships to assess the degree of homozygosity for loci under selection. Theor. Appl. Genet. **91:** 665–671.

Grundy, B., A. Caballero, E. Santiago and W. G. Hill, 1994 A note on using biased parameter values and non-random mating to reduce rates of inbreeding in selection programmes. Anim. Prod. **59:** 465–468.

Jansen, G. B., and J. W. Wilton, 1985 Selecting mating pairs with linear programming techniques. J. Dairy Sci. **62:** 1302–1306.

Kinghorn, B., 1987 On comparing strategies for mate allocation. J. Anim. Breed. Genet. **104:** 12–22.

Klieve, H. M., B. Kinghorn and S.A. Barwick, 1994 The joint regulation of genetic gain and inbreeding under mate selection. J. Anim. Breed. Genet. **111:** 81–88.

Lacy, R. C., 1989 Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. Zoo Biol. **8:** 111–123.

Land, A., and S. Powell, 1979 Computer codes for problems of integer programming. Ann. Discr. Math. **5:** 221–269.

Lush, J. L., 1946 Chance as a cause of gene frequency change within pure lines of livestock. Am. Nat. **80:** 318–342.

MacCluer, J. W., J. L. VandeBerg, B. Read and O. A. Ryder, 1986 Pedigree analysis by computer simulation. Zoo Biol. **5:** 147–160.

Mackay, T. F. C., 1985 A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*. Genet. Res. **47:** 59–70.

Meuwissen, T. H. E., 1997 Maximizing the response of selection with a predefined rate of inbreeding. J. Anim. Sci. **75:** 934–940.

Meuwissen, T. H. E., and J. A. Woolliams, 1994 Required effective sizes of livestock populations. Theor. Appl. Genet. **89:** 1019–1026.

Meyer, K., 1991 Estimating variances and covariances for multivariate animal models by restricted maximum likelihood. Genet. Sel. Evol. **23:** 317–340.

Meyer, K., 1993 DFREML user notes, v2.1. In ftp site: metz.enu.edu.au/pub/agbu/DFREML.

Misztal, I., and D. Gianola, 1987 Indirect solutions of mixed model equations. J. Dairy Sci. **70:** 1113–1121.

Nicholas, F. W., and C. Smith, 1983 Increased rates of genetic change in dairy cattle by embryo transfer and splitting. Anim. Prod. **36:** 341–353.

Nieto, B., C. Salgado and M. A. Toro, 1986 Optimization of artificial selection response. J. Anim. Breed. Genet. **103:** 199–204.

Nomura, T., and K. Yonezawa, 1994 The merits of mate selection with a limited maximum inbreeding coefficient for progeny animals. Anim. Sci. Technol. **65:** 647–651.

Partridge, L., and K. Fowler, 1993 Responses and correlated responses to artificial selection on torax length in *Drosophila melanogaster*. Evolution **47:** 213–226.

Perez-Enciso, M., and M. A. Toro, 1992 Classical and mixed-model analysis of an index selection experiment for fecundity in *Drosophila melanogaster*. J. Anim. Sci. **70:** 1060–1067.

Quinton, M., and C. Smith, 1995 Comparison of evaluation-selection systems for maximizing selection response at the same level of inbreeding. J. Anim. Sci. **73:** 2208–2212.

Quinton, M., C. Smith and M. E. Goddard, 1992 Comparison of selection methods at the same level of inbreeding. J. Anim. Sci. **70:** 1060–1067.

Robertson, A., 1961 Inbreeding in artificial selection programmes. Genet. Res. **2:** 189–194.

Robertson, F. W., 1957 Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. J. Genet. **55:** 428–443.

Rose, M. R., and B. Charlesworth, 1981 Genetics of life history in *Drosophila melanogaster*. Genetics **97:** 173–186.

Santiago, E., and A. Caballero, 1995 Effective size of populations under selection. Genetics **139:** 1013–1030.

Smith, S. P., and A. Mäki-Tanila, 1990 Genotypic covariance matrices and their inverses for models allowing dominance and inbreeding. Genet. Sel. Evol. **22:** 65–91.

Templeton, A. R., and B. Read, 1984 Factors eliminating inbreeding depression in a captive herd of Speke's gazelle. Zoo Biol. **3:** 177–199.

Toro, M. A., and B. Nieto, 1984 A simple method for increasing the selection response to artificial selection. Genet. Res. **44:** 347–349.

Toro, M. A., and M. Perez-Enciso, 1990 Optimization of selection response under restricted inbreeding. Genet. Sel. Evol. **22:** 93–107.

Toro, M. A., L. Silio, J. Rodrigañez and M. T. Dobao, 1988a Inbreeding and family index selection for prolificacy in pigs. Anim. Prod. **46:** 79–85.

Toro, M. A., B. Nieto and C. Salgado, 1988b A note on minimization of inbreeding in small-scale selection programmes. Livest. Prod. Sci. **20:** 317–323.

Toro, M. A., L. Silio and M. Perez-Enciso, 1991 A note on the use of mate selection in closed MOET breeding schemes. Anim. Prod. **53:** 403–406.

Van Tassell, C. P., and L. D. Van Vleck, 1996 Multiple-trait Gibbs sampler for animal models: flexible programs for Bayesian and likelihood-based (co)variance component inference. J. Anim. Sci. **74:** 2586-2597.

Verrier, E., J. J. Colleau and J. L. Foulley, 1994 Genetic variability when selecting on the animal model BLUP. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production IXX, Guelph, Canada, pp. 139–142.

Villanueva, B., and J. A. Woolliams, 1997 Optimization of breeding programmes under index selection and constrained inbreeding. Genet. Res. **69:** 145–158.

Villanueva, B., J. A. Woolliams and B. Gjerde, 1996 Optimum designs for breeding programmes under mass selection with an application in fish breeding. Anim. Sci. **63:** 563–576.

Wang, C. S., D. Gianola, D. A. Sorensen, J. Jensen, A. Christensen *et al.*, 1994 Response to selection for litter size in Danish Landrace pigs: a Bayesian analysis. Theor. Appl. Genet. **88:** 220–230.

Woolliams, J. A., and R. Thompson, 1994 A theory of genetic contributions. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production IX, Guelph, Canada, pp. 127–134.

Wray, N. R., and M. E. Goddard, 1994 Increasing long-term response to selection. Genet. Sel. Evol. **26:** 431–451.

Wray, N. R., and R. Thompson, 1990 Prediction of rates of inbreeding in selected populations. Genet. Res. **55:** 41–54.

Wray, N. R., J. A. Woolliams and R. Thompson, 1994 Prediction of rates of inbreeding in populations undergoing index selection. Theor. Appl. Genet. **87:** 878–892.

Wright, S., 1969 *Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies.* The University of Chicago Press, Chicago.

Communicating editor: R. G. Shaw